

AN INVESTIGATION INTO THE SECONDARY SWELLING
OF STARCH GRANULES

by

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TABLE OF CONTENTS

INTRODUCTION	1
LITERATURE REVIEW	1
Granular Structure and Physical Properties	1
Chemical Structure	9
Gelatinization and Granule Swelling	15
Retrogradation	24
Secondary Swelling	26
MATERIALS AND METHODS	31
RESULTS AND DISCUSSION	35
Appearance of the Secondary Viscosity Peak	35
Effect of Natural Lipids	37
Effect of Added Fatty Acids	42
Swelling Power and Solubility	43
Effect of Holding Temperature	44
Effect of Evaporation	49
Effect of Concentration	49
Enzyme Susceptibility	51
Effect of Carboxymethylcellulose	51
SUMMARY AND CONCLUSIONS	53
Proposed Mechanism	53
The Case of Potato Starch	56
ACKNOWLEDGMENTS	61
BIBLIOGRAPHY	62

INTRODUCTION

Starch is one of the most abundant plant carbohydrates in the world. It is the main reserve carbohydrate of the plant kingdom and a major part of the diet for man in nearly all parts of the world. It is unique among the plant carbohydrates in that it occurs in many parts of the plant in the form of crystalline granules. Besides its use as a source of energy for man and plant, starch, by virtue of its inherent properties and those which may be imparted to it by chemical or physical treatment, has found numerous uses in industry.

Of the natural products, few have been the subject of more intense investigation for so many years. Many different fields of study have contributed to our knowledge of the physical and chemical properties of starch, yet several very basic questions remain to be answered. One of the properties of starch which is important in its utilization in many areas is its ability to swell and absorb large amounts of water under the influence of heat. The purpose of this research project has been to study some of the phases of starch swelling and some of the factors which influence it.

LITERATURE REVIEW

Granular Structure and Physical Properties. Starch granules are found in all parts of the green plant, but mainly in seeds, where it provides reserve energy for the germinating embryo. It is also found in fruits, tubers, and in a transitory form, in the

leaves. Starch granules from different sources exhibit wide variations in size, shape, and other physical properties which make microscopic identification and characterization possible.

Granule size among different varieties is quite variable and may range from 2 microns to over 200 microns, measured over the longest axis. In spite of this large range, the size range is less and is relatively constant within a given species. Among commercial starches, rice starch granules are the smallest, with a range of from 2 to 8 microns, while potato starch granules, ranging in size up to 100 microns, are among the largest (36).

The shape of the granules depends not only on the variety of starch, but also, in the case of cereal starches, on its location within the kernel. Potato starch granules are ellipsoidal in shape, the small wheat starch granules are spherical, the large wheat starch granules are lens-shaped, and corn starch granules may be spherical if they are obtained from the loosely-packed floury endosperm or polygonal if they are obtained from the densely-packed horny endosperm (36). High amylose corn starch varieties may contain rod-shaped granules, branches or unbranched filaments, normal granules with various projections, and simple spherical granules (14, 18).

The tangential striations or lamellae are usually arranged concentrically around the hilum of the starch granule. In the naturally dried sample, the layers represent crystalline (starch rich) areas and amorphous (water rich) areas (6). The crystalline layers are birefringent, and retain this characteristic birefringence even after prolonged lintnerization in 7.5 percent

hydrochloric acid, while the amorphous layers do not show birefringence at all (3). It is thought (16) that the structure remaining after this acid treatment is mainly amylopectin, due to the fact that amylose is more easily hydrolyzed under these conditions. The lamellae are approximately 400 to 500 angstroms thick and there are between 50 and 100 lamellae in an average potato starch granule (16).

Badenhuizen (3) views the amorphous areas to be artifacts, produced by differences in the swelling of adjacent layers in alkali. The outer layer, being the first in contact with the alkali, swells first and leaves a hollow space between it and the next inner layer. The layers are formed during the synthesis of the granule, by a periodic mechanism which controls the deposition of the starchy material. The bonding between layers is necessarily weaker than that within layers, since it is due primarily to the penetration of molecules from the newly formed layer into the molecular network of the previously formed layer. Thus, the first manifestation of swelling in alkali is the separation of the granule into layers, and the greater the swelling of the granule the more conspicuous is the stratification. Careful dehydration of the naturally dried granule will cause the disappearance of the amorphous areas. It has also been reported that the layers become less conspicuous with increasing amylose concentration and molecular association (3).

It has been reported (5) that wheat starch granules grown under conditions of constant temperature, illumination, and moisture show no stratifications. However, Hess (21) and Roberts

and Proctor (58) have shown that potato starch granules grown under these constant conditions do produce stratifications. Hess (21) has measured the rate of formation of the layers in isolated potato starch granules and found that an average of two to three new layers were formed each day. He attributed this periodicity to an as yet unknown internal controlling mechanism.

The hilum is an open area within the granule. It is located either at the geometric center of the granule or eccentrically. Its actual location may be easily determined by observing the granule microscopically between crossed Nicol prisms when the hilum forms the center of the polarization pattern. If the hilum appears near the center of the granule, the pattern will be the familiar "Maltese cross." If it is located away from the center, the pattern may appear to be "V" shaped. The hilum is empty in the naturally dried cereal starches and is filled with liquid in the non-dried root and tuber starches (36).

Starch granules are birefringent. This phenomenon, which is caused by the radial arrangement of optically positive polymers, is characteristic of spherocrystalline substances. According to Badenhuisen (3), tuber starches generally show stronger birefringence than do cereal starches, and high amylose cereal varieties show the weakest birefringence of all. This author has pointed out that the stronger the molecular association is, the weaker will be the birefringence. It is thought (3) that the branched fraction contributes most to birefringence, because of its limited deformability and consequently stronger radial orientation. The linear fraction is more easily deformed, and its

radial orientation may therefore be lessened. This view is supported by the fact that after prolonged acid treatment the birefringence is still evident in the residue which is composed mainly of amylopectin (16).

Starch granules, when heated 10 to 15°C above the temperature at which birefringence is lost, may be partially returned to the original spherocrystalline state by the addition of weak iodine solutions, ethanol, or pinacyanole (35). This suggests that the disorganization of the crystalline structure is not complete even after all birefringence is lost.

Birefringence may also be destroyed by physical damage to the granule. Crushing the granule through the application of shearing forces destroys the crystalline structure of the granule, resulting in a loss of birefringence. However, a granule may be carefully sectioned without any such loss of birefringence (38).

On a molecular level, the internal structure of the starch granule is described by the model originally proposed by A. Meyer (42) in 1881 and further modified by K. H. Meyer in 1942 (43). A. Meyer (42) proposed that the granules were built up of crystals of starch substance which are densely packed in concentric layers. Intermediate layers separate these and consist of the crystals in a less densely packed form. According to K. H. Meyer (43), the linear and branched molecules are radially oriented in the granule. Wherever linear segments of the branched molecules or linear molecules are parallel to one another, hydrogen bonding between the segments links them together, forming crystalline

areas or "micelles." A single branched molecule may be associated with several micelles through its many terminal branches, and similarly, a single linear molecule, because of its relatively great length, may also associate with or tie together several micelles. Thus, the internal structure is assumed to be not a random conglomeration of amylose and amylopectin molecules, but an ordered array of crystalline micelles.

The occurrence of hydrogen bonds and their importance in explaining the physical properties of starch granules have been extensively reviewed by Caesar (10). It is that author's view that hydrogen bonding is the most important aspect of granular structure. The forces acting to hold the micelles together are principally hydrogen bonds. In naturally dried cereal starches, these bonds are formed between adjacent chains with water molecules acting as intermediates. In drastically dried starches, which seem to lose intramolecular water, these intermediates are lost and the bonds are formed directly between adjacent chains. The principal bonding probably occurs at the hydroxyl group on the sixth carbon atom of the glucose residue, with secondary bonding occurring at the hydroxyl groups on the second and third carbon atoms (10). Mullen and Pacsu (52) have studied the heat of gelatinization of several starches and the results indicate that these heats of gelatinization are of an order of magnitude corresponding to the energy required for one or two hydrogen bonds per glucose residue. The degree of association in a granule depends on the number of these hydrogen bonds, and is affected by the spatial relationship of the

hydroxyl groups on one molecule to those on an adjacent molecule. The great tendency of the linear fraction to retrograde is thought to be associated with the ease of formation of large numbers of these hydrogen bonds due to the readiness with which parallel arrangement of these molecules occurs, while the spatial problems involved in the close alignment of the branched fraction are reflected in its much lower tendency to retrograde.

Ulmann (73) has shown that the water of crystallization held by a single glucose residue may vary from one to five molecules, with the moisture content of the granule varying from 10 to 35 percent. This water is necessary for the orientation of the chains in the crystalline network, since anhydrous or strongly hydrated granules are amorphous.

Sair and Fetzer (61) have shown that the amount of sorbed water is inversely related to the degree of molecular association. While the amount of water held increases in the series cereal-root-tuber, the degree of molecular association decreases in the same order. Badenhuizen (3) has stated that the degree of association depends on the hydrogen bonding between hydroxyl groups and that the number of free hydroxyl groups depends on the amount of water present during the formation of the granule. As less water is available during this period, the number of free hydroxyl groups available for direct hydrogen bonding increases and this has the effect of increasing the molecular association.

The distribution of amylose and amylopectin within the granule has been investigated, but the evidence is inconsistent. Czaja (13) stated that the amylopectin fraction was concentrated

at the periphery of the granule. Baker and Whelan (4) found that several varieties of waxy starches exhibited a central core which stained deep blue upon treatment with iodine solution while the periphery of the granule stained purple or reddish-purple. The position of this core was found to correspond to the intersection of the arms of the polarization cross. The size of the core was dependent on the amylose content of the sample and it was concluded that a gradient in the amylose concentration existed from the center of the granule to the periphery (4). The small amount of amylose present in waxy maize starch is concentrated in a central nucleus, which stains blue in the presence of iodine after the granule has been lintnerized for 30 days, while the rest of the granule retains a yellow color (2). This author found no evidence of a similar gradient in potato starch nor in wrinkled pea starch. Leach and Schoch (34), in their study of enzymatic digestion of raw granules, found that the iodine affinity of the residue after digestion was the same as that of the parent granules and they concluded that the fractions were uniformly distributed throughout the granule. Wolf et al. (80) have recently shown that the irregularly shaped starch granules from high amylose corn starch show differential iodine affinities; some parts of these irregular bodies absorb iodine while other parts of the same granule do not. This differential staining was attributed to either variable distribution of amylose and amylopectin or differences in the degree of molecular association in various sections of the granule.

The strength of the intermolecular bonds may be constant throughout the granule or they may vary greatly (33). Potato starch, for example, swells uniformly within a small temperature range, indicating a single set of uniformly weak bonds. Unmodified corn starch, on the other hand, undergoes rapid swelling between 60° and 70°C and again between 85° and 95°C. This led to the conclusion (33) that there are two sets of bonding forces present, both of which are stronger than those of potato starch. Starches with a high percentage of the linear fraction show greatly restricted swelling even at 95°C, indicating very strong associative forces (33).

Chemical Structure. Starch is composed of two major polysaccharide components, amylose and amylopectin. There is some evidence for a third distinct fraction, with properties intermediate between the strictly linear amylose and the highly branched amylopectin, which comprises approximately 5 to 7 percent of the total starch material (32, 56). The two main fractions occur in a genetically fixed ratio which varies in starches from different sources. Waxy varieties of corn, rice, sorghum, and barley are composed almost exclusively of amylopectin. Others such as normal corn starch, potato starch, and wheat starch contain between 17 and 29 percent amylose, the remainder being amylopectin. At the other extreme are wrinkled pea starch, which contains approximately 60 to 70 percent amylose, and high amylose corn starches, which contain between 55 and 80 percent amylose.

The amylose component of starch is a relatively low molecular weight polysaccharide consisting of some 200 to 700 glucose

units (15). Methylation studies on amylose indicate that there is approximately one non-reducing residue per 200 glucose units, and that the products of hydrolysis after methylation are 2,3,6-trimethyl glucose and approximately 0.3 percent of 2,3,4,6-tetramethyl glucose. These results indicate that amylose is a linear molecule composed of glucose units linked exclusively through the 1 and 4 positions, with one free aldehyde group per molecule (47).

Amylose is a heterogeneous fraction with widely varying chain lengths and molecular weights. Kerr et al. (30) have shown that the butanol-precipitated fraction may be further fractionated to yield subfractions with chain lengths of from 250 to 675 glucose units. Other properties of these various subfractions, such as intrinsic viscosity, the alkali labile number, and the ferricyanide reducing number, also vary (30).

Amylose is distinguished by its ability to complex with iodine to produce a deep blue color. In the "V" X-ray configuration amylose is capable of absorbing up to 25 percent or more of its weight in iodine (15). The reaction occurs with dry amylose, obtained by precipitation with one of a number of complexing agents, in the presence of iodine vapor, and with amylose solutions in the presence of iodine and triiodide ions. Amylose in the dry granule, or retrograded amylose, does not possess the proper helical configuration and has, therefore, a low iodine affinity (15). The native granule in a moist condition will take up iodine, indicating that there are free amylose chains within the granule capable of assuming the proper configuration (15).

Rundle and Baldwin (59) have shown that amylose-iodine solutions exhibit dichroism during flow. This finding suggested that the long axes of the iodine molecules were oriented parallel to the direction of flow, or parallel to the long axes of the amylose molecules. This interpretation was later confirmed by Rundle and French (60) in a study of the optical properties of Schoch's butanol-precipitated fraction and Kerr's crystalline amylose (30). The results indicate that the proposed model in which the iodine molecules are held within the amylose helix satisfactorily explains the observed optical properties of the system.

Osman et al. (54) have investigated the effect of various surface active agents on the iodine affinity of amylose and have shown that the reduction in iodine affinity depended to a certain extent on the type of surfactant employed. X-ray patterns of the surfactant-amylose complexes were comparable to those of fatty acid-amylose complexes, indicating that the same type of complex was formed. Mono-, di-, and triglycerides gave comparable patterns, which led to the conclusion that only one of the fatty residues of the surfactant was involved in the complex.

All of the mono- and diglycerides considered in the above study reduced the iodine affinity below 5 percent from an original value of 15.2 percent (54). Diglycerides from soybean oil reduced it only to 11.7 percent, while whole soybean oil had little effect. With all fatty materials studied, a minimum iodine affinity was reached and further addition of surfactant had no effect.

It was also shown by these investigators (54) that a linear relationship exists between the amount of surfactant added and the iodine affinity. These straight line relationships could be extrapolated to 0 percent iodine affinity, and it was found that for surfactants with a single hydrocarbon chain the amount of surfactant necessary to reach 0 percent iodine affinity was inversely related to the length of its hydrocarbon chain.

Bourne et al. (9) have reported that addition of a 1 percent sucrose stearate solution to a gelatinized starch suspension results in the precipitation of up to 20 percent of the starch material at stearate concentrations above 0.06 percent. Fractionation of the starch and treatment of the separated fractions with the stearate showed that much of the linear portion was precipitated, whereas little of the branched fraction was. Controlled acid hydrolysis of potato starch and subsequent precipitation with stearate indicated that the molecular weight of the linear fraction was an important factor in determining whether a precipitate would form and also the amount of precipitation.

Schoch and Williams (70) have demonstrated that removal of fatty material from corn starch raised the iodine affinity of the starch. Addition of palmitic acid to the butanol-precipitated fraction lowered the iodine affinity progressively as the concentration of the acid was increased. This led to the conclusion that the naturally occurring fatty materials in corn starch were present as the amylose complex.

The linear amylose molecules are also able to complex with a wide variety of organic molecules. Bear (7) has reported that

insoluble complexes were formed with linear and branched chain alcohols, ketones, aldehydes, and fatty acids. These complexes depend on the ability of amylose to form a helical or "V" type X-ray configuration. The diameter of the helices is variable, depending on the particular complexing agent, but only certain diameters are possible. The compounds known to form complexes have in common the possession of a permanent dipole moment or the ability to form an induced moment. These complexes are thought to be of the same type as the amylose-iodine complex in which the complexing agent is situated within the helical amylose molecule. Mikus et al. (48) have studied the various amylose complexes in detail, and have shown through X-ray studies that the distance between helices in the "V" type crystals and in fatty acid complexes is the same. It was concluded that the fatty acid had to be within the helix and not between helices. It was also shown (48) that upon heating the fatty acid-amylose complex in the presence of iodine vapor, the fatty acid was replaced by the iodine, indicating that they both occupied the same position within the amylose helix.

The ability of amylose to complex and precipitate in the presence of various organic compounds has been utilized in the fractionation of whole starch into amylose and amylopectin. The first quantitative fractionation procedure was developed by Schoch (64, 66) using butyl and amyl alcohols, and independently by Wiegel (78, 79) using ethyl and butyl alcohols. Schoch and Williams (70) demonstrated that fatty acids could be used as fractionating agents because of the formation of a fatty

acid-amylose complex. MacMasters et al. (37) showed that fatty acid-amylose complexes were formed spontaneously in starch pastes held at elevated temperatures, if fatty acids or soaps were present. Whistler and Hilbert (77) utilized nitroparaffins, pyridine, mercaptans, and other compounds which possessed either donator or acceptor groups capable of hydrogen bonding. Hiemstra et al. (23) have used inorganic magnesium salts to precipitate amylose, this method being based on the familiar "salting out" effect used extensively in protein fractionation. Other separation techniques include cellulose adsorption of amylose (55) and in the case of potato starch, the presence of phosphate groups allows the utilization of electrosedimentation techniques (62).

The second major starch fraction, amylopectin, is a high molecular weight polysaccharide, with values ranging between 100,000 and 1,000,000, depending on the source of the amylopectin, isolation techniques, and the method used in determining the molecular weight (43). In contrast to the linear character of amylose, amylopectin is a highly branched molecule, its longest branches being only about 20 to 30 glucose residues in length. The amylopectin of cereal starches does not contain any chemically bound phosphorous (57) but that of potato or arrow root do contain small amounts as phosphoric esters at the hydroxyl group on the sixth carbon atom of the glucose residue (46, 47). Amylopectin, upon methylation and hydrolysis yields 91 percent of 2,3,6-trimethyl glucose, 4 percent of 2,3,4,6-tetramethyl glucose, and 5 percent of 2,3-dimethyl glucose. The branches are linked solely through 1,6 glucosidic bonds (17). Enzymatic degradation

with beta-amylase indicates that approximately 65 percent of the glucose residues are located in the outer branches of the molecule, that is, beyond a 1,6 branching point.

Wolff et al. (81) and Montgomery et al. (50) have shown that the amylopectin isolated from amylo maize varieties exhibit properties which indicate a greater degree of linearity than amylopectin from normal starches. This fraction exhibited a higher iodine affinity, was less soluble, had a lower degree of polymerization, and showed a greater tendency to retrograde than normal amylopectin (81). It was concluded that both the interior and terminal branches of this amylopectin fraction were longer than those in normal amylopectin, giving the molecule properties intermediate between amylose and normal amylopectin (81).

As a result of the reduced length of the free terminal chains, amylopectin has a low iodine affinity. In contrast to the deep blue color produced by amylose with iodine, amylopectin gives only a red or purple color. Amylopectin does not form complexes with organic compounds as does amylose and has a lower conversion limit with beta-amylase. Another consequence of its shorter terminal branches and bushy structure is its low tendency to retrograde.

Gelatinization and Granule Swelling. Granular starch is insoluble in cold water, not over 0.1 percent dissolving in distilled water at room temperature. Even this small amount of soluble material is usually attributed to the solubility of damaged granules. If an aqueous starch suspension is heated above a certain critical temperature range, which is

characteristic for a given starch, the granules undergo irreversible hydration and swelling, to form a paste. As the temperature is raised above the critical level, swelling and solubilization continue with a consequent weakening of the granular structure and an increased susceptibility to fragmentation under mild mechanical treatment.

The pasting process may be conveniently divided into three stages. Upon initial contact with cold water, limited and reversible hydration and swelling occur. At this point, there is no perceptible change in viscosity, and the birefringence is not affected. The starch, in this condition, may be dried with no alteration in its original properties. Upon further heating, the second stage is entered in which the granule swells greatly and absorbs water irreversibly. Birefringence is gradually lost, from the hilum outward, over a range of a few degrees, and the viscosity increases more or less continually as the temperature increases to boiling. Much of the amylose is solubilized and diffuses into the supernatant liquid and the granules cannot be returned to their original state by drying. During the third stage of gelatinization the granules appear to be formless sacs enclosing a vacuole which is filled with dissolved starch. These sacs are quite weak and are easily fragmented by mechanical agitation, but do not burst of their own accord.

The formation during the final stage of gelatinization of the granule sac led early investigators to believe that it was a preformed part of the granule. It now appears that the sac is produced during the gelatinization process by the fusion of the

amylopectin layers as they swell (3, 74). It has been shown that removal of the outer layers of starch granules through digestion with salivary amylase does not affect the mode of granule swelling nor prevent the appearance of the granule sac (34).

On a molecular level, gelatinization is thought to involve the entrance of water into the crystalline network of the granule, and, under the influence of heat, a subsequent weakening of the hydrogen bonds which hold the micellar areas together. Concurrent with the relaxation of the micellar hydrogen bonds, there occurs a general breakdown of the water molecule aggregates under the influence of heat, which effectively increases the penetrating power of the water. The initial swelling occurs in those areas where the intermolecular bonds are weakest, in the amorphous or non-micellar areas. The linear molecules may become partly detached in these areas and the shorter linear molecules diffuse out of the swollen granules. Eventually, a quantitative removal of amylose is possible at 85°C, if the supernatant liquid is constantly changed (45). As more energy is added, the bonds in the more strongly associated areas are relaxed and these areas swell. Coincident with the destruction of the crystalline structure of the granule birefringence is lost, beginning at the hilum and proceeding to the periphery. This loss occurs over a range of several degrees. Birefringence may return under certain conditions, indicating that the crystalline structure is not completely destroyed, even after complete loss of birefringence (35).

Starch granule swelling is influenced by many factors other than the source of the starch. Granule size, the percentage of

the linear fraction and degree of association, the final temperature, the rate of heating, mechanical damage, inorganic and organic additives, and chemical treatment, all affect swelling to a certain extent.

The rate of heating will affect the degree of swelling in a given sample (3). Starch heated slowly in water will show reduced swelling due to retrogradation or aggregation of the linear fraction within the granule. More energy is necessary to relax the additional hydrogen bonds formed through this retrogradation and a higher temperature is necessary to attain the same degree of swelling as in a rapidly heated sample.

Introduction of cross bonds, through chemical treatment of the starch with a metaphosphate or epichlorohydrin, decreases the swelling of the granules as well as the amount of soluble material leached from the granule (41). Treatment of the starch with acid causes breakage of the hemiacetal linkages between units in the starch molecules, resulting in a shortening of the chains and greater solubilization and fragmentation in hot water (36).

Bourne et al. (9) have reported that the addition of 1 percent sucrose stearate to starch suspensions caused a reduction in the rate at which the starch granules swelled.

Marnett and Selman (40) have shown that polyoxyethylene monostearate produces an inhibition of alpha-amylase activity through a modification of the starch substrate. It was concluded that the monostearate acted mainly by inhibiting granular swelling, thereby reducing the amount of soluble material leached from it.

The volume of sediment which could be settled out of wheat starch slurries after heating to various elevated temperatures was shown by Stradine et al. (71) to be decisively influenced by the type and amount of fatty material present. The volume increased with increasing temperature and decreasing amounts of monoglycerides. The amount of amylose leached from the granule also followed the same trends. The presence of monoglycerides further decreased granular swelling, hydration, and inhibited the loss of birefringence. Granular fragmentation was also reduced, possibly due to decreased swelling.

Osman and Dix (53) reported that increasing amounts of fat in a 6 percent corn starch paste caused the initial viscosity increase to occur at progressively lower temperatures, with the temperature at maximum hot paste viscosity also being lowered. These changes occurred until a fat concentration of 9 to 12 percent was reached, after which no further changes were noted. A characteristic step in the cooling curve was also eliminated at concentrations as low as 0.2 percent fat. Ten natural and hydrogenated fats were studied with essentially the same results.

When surface active agents were added to the starch-water-fat slurry, the changes in the viscosity curves varied (53). In general, the temperature at which the initial viscosity increase occurred was raised, and the shape of the curves was appreciably altered. The magnitude of the maximum viscosity remained constant, but the temperature at which it occurred was influenced by the type of surface active agent employed. This temperature was raised with increasing chain length in monoesters and, to a

lesser extent, with diesters also.

The effect of various surfactants and fatty acids on the swelling and solubility of starches has been investigated by Gray and Schoch (20). The results indicate that surfactants with hydrocarbon chains of four or five carbon atoms enhance the hydration of corn starch, causing a decrease in the gelatinization temperature and an increase in the amount of soluble material. Those surfactants with hydrocarbon chains of about 12 carbon atoms tend to complex with the linear fraction, inhibiting swelling and solubilization of the starch below 85°C; above this temperature, the complex breaks down and the surfactant may act to enhance hydration, causing increased swelling and solubilization. Surfactants with hydrocarbon chains of 18 carbon atoms form complexes with the linear fraction which are stable up to 95°C, hence they repress swelling and solubilization over the entire temperature range investigated.

Sodium lauryl sulfate, containing a hydrocarbon chain of 12 carbon atoms, repressed swelling and solubilization of corn and potato starch up to 85°C (20), at which point the inhibition was reversed and the surfactant acted to enhance both swelling and solubility. With waxy sorghum starch, which contains little or none of the linear fraction, swelling and solubility were enhanced over the entire temperature range. Cationic quaternary ammonium surfactants enhanced swelling and solubility of corn and waxy sorghum starches with no evidence of complexing. These ammonium surfactants had just the opposite effect on potato starch, repressing swelling and solubility over the entire

temperature range. This was attributed to the combination of the phosphate groups of potato starch with the cationic quaternary ammonium groups of the surfactant, inhibiting swelling and solubility through the formation of van der Waals forces. Polyoxyethylene monostearate gave evidence of complexing with the branched fraction, since it repressed swelling of both normal and waxy starches.

The effects of stearic acid and its homologs were studied in greater detail (20). Increasing amounts of stearic acid were added to potato starch in a manner designed to regenerate the fatty acid within the granule. Amylograph curves were obtained for these samples which showed that the increase of fatty material elevated the initial gelatinization temperature and maximum hot-paste temperature, decreased the maximum hot-paste viscosity, lowered the degree of breakdown after reaching the maximum viscosity, and increased the cold-paste viscosity. There was also a decrease in the swelling power and extent of solubilization.

The length of the hydrophobic chain also affected the amylograph curves, swelling power, and solubilization (20). Addition of 1 percent fatty acid, in the homologous series from caprylic to arachidic acid, to potato starch caused a decrease in swelling power and solubility through myristic acid, then an increase in both. This indicates that myristic acid is the most effective of the series in forming the fatty acid-amylose complex.

The results of this study were interpreted on the basis of the formation of a fatty adjunct-amylose complex (10). If the swollen starch granule is assumed to be a loose network of

extended linear molecules interspersed with expanded branched molecules, held together by hydrogen bonding, then in the presence of a fatty adjunct the formation of a fatty acid-amylose complex would cause the extended amylose molecules to assume a helical configuration, thereby effectively tightening the molecular network. The total effect of this action would be to reduce the swelling power of the granules and also to reduce the extent of solubilization.

Hester et al. (22) have shown that addition of sucrose to corn and wheat starch slurries affects the swelling, solubility, and gel properties of the pastes. The temperature at which the initial swelling occurred increased as the sucrose concentration was increased in the case of corn starch, but no such change was reported for wheat starch. Other characteristics of the amylograph curve were not affected by the addition of sucrose. The amount of soluble material in the supernatant after centrifugation of the suspension was decreased by the addition of sucrose. The results obtained with corn starch suggested a decrease in the initial and final degree of hydration of the starch. Microscopic studies were inconclusive as to whether or not there was a decrease in granular swelling with the addition of sucrose. Increasing the sucrose concentration generally caused an increase in the gel strength of the pastes.

Studies on the action of cold gelatinizing agents have shown that their main function is to cause the disassociation of the water molecule aggregates into smaller aggregates, and eventually into single water molecules, thereby increasing the penetrating

power of the water (10). The water molecule aggregates are held together by hydrogen bonds, which are weakened by the high dielectric constant of aqueous solutions of these cold gelatinizing agents. Heat also causes disassociation of the water molecule aggregates through destruction of the hydrogen bonds. Thus, heat may cause granular swelling in two ways, by destruction of the water molecule aggregates and by relaxation of the hydrogen bonds within the starch granules (10).

Many inorganic compounds have been found to be effective cold gelatinizing agents. Hodge et al. (24) reported that liquid ammonia at -35°C caused swelling and partial disorganization of the crystalline structure of the starch granule, with a loss in birefringence. Mangels (39) has shown that sodium hydroxide, potassium thiocyanate, sodium salicylate, and urea, may act as cold gelatinizing agents, and that the degree of swelling produced increases with increasing concentration of the agent. Schaer (63) reported that chloral hydrate, under conditions of low concentration, caused gelatinization of starch granules. At higher concentrations, it produced no gelatinization and this was attributed to the lack of sufficient water for the hydration of the starch.

Gortner (19) concluded that cold gelatinization through the action of chemicals was not the same phenomenon as heat gelatinization, although all cold gelatinizing agents seem to have the same mode of action. This conclusion was based on the fact that the volume occupied by a cold-gelatinized sample was approximately twice that occupied by a similar, heat gelatinized sample. It

was shown that the extent of cold gelatinization depended on the concentration of the agent employed. At low concentrations, no hydration occurred. As the concentration increased, a rapid swelling of the granules was observed, ending in the rupture of the granule if the concentration was increased to a high level. When the concentration was raised too high, dehydration of the starch became evident.

Retrogradation. Upon standing at or below room temperature, amylose solutions and starch pastes undergo several changes. An amylose solution becomes progressively more turbid, eventually yielding a microcrystalline precipitate. This precipitate gives a "B"-type X-ray pattern and under certain conditions may show birefringence (15). Because of the conformation of the amylose chains and their position within the crystal, the iodine affinity of amylose in this condition is greatly reduced, as is the enzyme susceptibility. Starch pastes which have undergone retrogradation set up into firm gels.

The changes which take place on a molecular level have been attributed to the formation of hydrogen bonds between hydroxyl groups on adjacent amylose and amylopectin molecules (10). At temperatures below the gelatinization temperature, more of these bonds are formed than are broken at any instant, resulting in a net increase in the number of these bonds and a closer association of the participating molecules.

The branched configuration of the amylopectin molecule and the relatively shorter length of its terminal chains are probably not as conducive to close alignment and formation of hydrogen

bonds, resulting in an easier dispersion of the molecules and greater reversibility of retrogradation. Amylose, on the other hand, with its linear configuration, would permit many such bonds to form, and create a close and strong association between molecules. This is reflected in the difficulty encountered in dispersion of retrograded amylose, which can be accomplished only by heating the retrograded material to 150°C or by the addition of strong alkali.

Holló et al. (27) have studied the physico-chemical relations involved in retrogradation. These authors divided the process into the following three stages. First, addition of energy to cause the randomly coiled amylose chains to be extended. The energy necessary for this is supplied by the formation of hydrogen bonds. The second step is the arrangement of these chains parallel to one another, after the loss of the water of hydration shell. The third step is the formation of hydrogen bonds between adjacent hydroxyl groups. When a great number of molecules has become associated in this manner, the solution becomes turbid and eventually a precipitate is formed.

Some of the factors which influence retrogradation have also been studied by Holló et al. (26). The rate of retrogradation increases from a minimum at 60°C to a maximum near 0°C . A maximum is also observed at pH 7. Inorganic salts increase the rate of retrogradation through aiding in the dehydration of the molecules to produce the "salting out" effect. The degree of polymerization of the amylose also exerts an effect on the rate, with minimum values being obtained at very high and at very low

degrees of polymerization. This effect is attributed to the steric difficulty involved in aligning very long molecules and the increased solubility of very short ones. Addition of crystals of retrograded material to amylose solutions also increases the rate, as does simply increasing the concentration of the solution.

Secondary Swelling. The degree of swelling which a granule attains in excess water becomes primarily temperature dependent. For potato starch and many waxy starches this dependence is linear over the entire range of temperatures during which swelling occurs. For other starches, such as unmodified corn starch, sorghum starch, and barley starch, the relationship is definitely non-linear. The temperature vs. degree of swelling curves show ranges with rapid swelling and others with limited swelling (33).

Katz and Rienstma (29) have reported on the changes which occur in the X-ray spectrum of wheat starch as it is heated in excess water to 100°C. Untreated wheat starch granules exhibit the characteristic A-type spectrum. The first change in the spectrum occurs as the sample is heated in excess water to about 62.5°C, at which point the granules lose their birefringence and swell and the spectrum changes to a "V" or amorphous type. This amorphous spectrum is retained to a temperature of approximately 100°C, at which point it changes again. The change from the "A" to the "V" spectrum can also be obtained by heating the sample to 100°C with a limited amount of water.

Katz (28) has also shown that changes in the solubility and swelling power of wheat starch correspond to changes reported in

the X-ray spectra. A rapid increase in the swelling power and solubles occurs between 55° and 65°C. They continue to increase at a reduced rate until a temperature of 80°C is attained, at which point both show again a rapid increase. Between 80° and 90°C, both swelling power and solubles are approximately doubled.

The amount of water available to the starch also limits the degree of swelling and of solubility (28). The swelling power and solubility show minimum values in an air-dried sample which is heated to 100°C and held for one hour. Both increase slowly as the moisture available during the heating increases to 50 percent. Between 50 and 60 percent moisture a rapid increase in both solubility and swelling power occurs. Other rapid increases in swelling power occur when the moisture level reaches 700 and 1200 percent, without corresponding increases in the solubility.

Light transmission techniques have been applied to the study of starch gelatinization by Morgan (51), Cook and Axtmayer (11), and Beckford and Sandstedt (8). It has been shown (8) that wheat, rye, and unmodified corn starches, all undergo two-stage swelling, whereas potato and navy bean starches undergo only a single stage. When wheat starch was separated into large and small granule fractions both exhibited the same two-stage swelling as the parent starch. The concentration of starch in the suspension was critical, since the second stage was not evident at either very high or very low concentrations.

Crossland and Favor (12) studied gelatinization with the amylograph and demonstrated that the sensitivity of the method could be increased by suspending the starch in a viscous,

water-binding medium such as aqueous solutions of sodium alginate or carboxymethylcellulose. With these media it was possible to show the stages in the viscosity curves of wheat and corn starches, but not in wrinkled pea, potato, waxy maize, or tapioca starches.

The addition of sodium alginate greatly reduced the apparent gelatinization temperature of wheat starch pastes, with increasing amounts causing progressive delineation of the two stages (12). The shape and characteristics of the curves obtained with the alginate and with carboxymethylcellulose were similar, indicating that the effect is not due to a chemical reaction of the starch with the specific colloid.

Carboxymethylcellulose has also been employed to demonstrate the stages in the swelling pattern of barley starch (18).

It has been shown (49) that if rye starch is heated in the presence of lauric acid at 90°C and held at this temperature, a second viscosity peak appears. Waxy sorghum exhibited no secondary peak under the same conditions, leading to the conclusion that the secondary peak is caused by the amylose fraction of the starch. The presence of fatty acids increased the gelatinization temperature and inhibited granular fragmentation and breakdown. The secondary peak was attributed to either a greater degree of hydration in the presence of fatty acids or to the mutual adherence of the granules, favored by the inhibition of disintegration and increase in the amount of water held by these aggregates.

Leach *et al.* (33) have shown by means of solubility and swelling power studies that corn starch undergoes two distinct

stages in its swelling and solubility curves. The first stage occurs within the usual gelatinization temperature range of about 60° to 67°C and is accompanied by a loss of birefringence and an irreversible uptake of water. As the temperature increases further, both swelling power and solubles increase at a reduced rate until a temperature of 85°C is reached, at which point both solubles and swelling power exhibit a marked increase. Other starches which exhibited this two-stage pattern were milo and waxy milo, while potato and tapioca starches exhibited only single-stage patterns.

Starches containing a high percentage of the linear fraction showed reduced swelling power and solubility, even at 95°C (33). Wrinkled pea and high amylose corn starches gave swelling powers of 5.5 and 6.3, respectively, at 95°C, compared with a value of 18 for unmodified corn starch at 90°C. This reduction was thought to result from a much greater degree of molecular association in the high amylose samples.

Mazurs, Schoch, and Kite (41) have reported two instances of increased viscosity upon prolonged cooking of starch pastes. A cross-bonded waxy sorghum starch cooked for one hour at 95°C exhibited an increasing viscosity trend over the entire cooking period. This increasing trend was attributed to the gradual relaxation of the cross bonds and the very low degree of granular fragmentation. The cross-bonded starch exhibited reduced swelling and solubility.

The other instance reported by these authors (41) was that of an 80-fluidity acid modified corn starch. This sample

exhibited a hot-paste peak and subsequent breakdown, reaching a minimal viscosity at about 93°C. Upon prolonged cooking at 95°C, the viscosity showed a great increase and remained high over the entire one-hour cooking period, with only slight decreases at higher concentrations. As there is a great degree of granular disintegration in this acid-modified starch the phenomenon was attributed to the formation of an unstable, molecularly dispersed solution. At high concentrations this instability resulted in the formation of a gel, which was subsequently broken down by the mechanical agitation in the amylograph.

Watson (76) has shown that a secondary viscosity peak is exhibited by unmodified corn and wheat starches, and that high amylose corn starch shows a continually rising secondary viscosity increase if the sample is suspended in water and held at elevated temperatures for long periods. Waxy corn starch does not show a secondary peak.

Increasing the holding temperature increased the initial hot-paste viscosity, the degree of breakdown after the initial peak, and the time required to reach the secondary peak (76). The height of the secondary peak relative to the plateau viscosity also decreased with increasing holding temperature. Removal of the fatty materials from unmodified corn starch by a 24-hour Soxhlet extraction, using 80 percent methanol as the solvent, caused little change in the secondary peak characteristics (76).

Addition of carboxymethylcellulose caused an increase in the initial viscosity and decreased the time necessary to attain a

secondary peak. This was in agreement with the results reported by Crossland and FAVOR (12).

The secondary peak was attributed to the formation of an amylose-amylose complex (76). It was thought that after being leached from the swollen granules, the amylose molecules were aligned by the action of the amylograph and hydrogen bonds were formed between the adjacent chain segments, resulting in the formation of a molecular network. The subsequent viscosity decrease was thought to be brought on by a breakdown of this network, caused by the mechanical agitation of the amylograph.

MATERIALS AND METHODS

Six commercially prepared starches, including unmodified thick-boiling corn starch, waxy corn starch, potato starch, wheat starch, grain sorghum starch, and waxy sorghum starch were employed in this study. The corn starches were obtained from American Maize Products Company, potato starch and the sorghum starches from Corn Products Refining Company, and the wheat starch from Hercules Powder Company. Carboxymethylcellulose, CMC-7HP, was obtained from Dow Chemical Company.

Amylograph viscosity curves were obtained for each of the various samples by suspending appropriate amounts of the starch, on a dry basis, in 460 ml of distilled water and transferring this suspension quantitatively to an amylograph bowl. All amylograph studies were begun at 50°C and the temperature was increased at a constant rate of 1.5° per minute, to the final holding temperature. Separate determinations were made with the top

cooler in operation, in order to determine the effect of evaporation on the viscosity characteristics. When carboxymethylcellulose was used, 4.6 g of this colloid and 350 ml of distilled water were mixed and homogenized in a blender, transferred quantitatively to the amylograph bowl, and held at 45°C until a constant viscosity was attained. The appropriate amount of starch was suspended in 110 ml of distilled water and this suspension transferred quantitatively to the amylograph bowl; the temperature was again held at 45°C until constant viscosity had been reached. The temperature was then raised in the normal manner. The final concentration of carboxymethylcellulose in these suspensions was 1 percent.

The two methods described by Schoch (68) were used to defat individual portions of the starch samples. In the bulk method, 300 g of starch and 900 ml of 85 percent methanol were refluxed in a 3-liter, three-necked flask, fitted with a reflux condenser, a stirring mechanism, and a heating mantle. After refluxing for two hours, the suspension was filtered while still hot and washed with fresh, hot solvent. The cake was broken up and resuspended in fresh solvent and the operation repeated. Each sample was subjected to a total of four such cycles. After the final reflux, the cake was suspended in distilled water and stirred for 30 minutes to remove the last traces of alcohol. After a final washing in distilled water the suspension was filtered, broken up, and allowed to air dry for 48 hours.

Fifty gram samples of unmodified corn starch were also defatted in a Soxhlet extraction apparatus for 24 hours, using 85

percent methanol as the solvent. After extraction, these samples were washed in distilled water and treated in the same manner as the bulk defatted samples. Moisture determinations were run on all samples by drying 2 g samples for four hours at 110°C in a vacuum oven.

The starches were refatted by a procedure designed to regenerate the fatty acid as it naturally appears in the granule (20). The desired amount of potassium oleate was added to a starch-water mixture, which was thoroughly stirred and immersed in a water bath at 50°C. The suspension was stirred continuously and held at this temperature for four hours. It was then filtered and the cake washed with distilled water to remove any excess soap. The starch was resuspended in distilled water and the pH adjusted to 5 with dilute hydrochloric acid in order to regenerate the fatty acid. The slurry was then refiltered and washed several times with distilled water until a negative test for chloride was obtained. The filtered and washed cake was then broken up and allowed to air dry.

Microscopic examinations were conducted on several samples of starch. Small samples were removed from the amylograph bowl at appropriate intervals and diluted with hot distilled water at the same temperature as that of the suspension in the amylograph. By use of a micrometer eyepiece and a hemocytometer slide, granule sizes were determined for 250 granules in each sample. The number of broken granules and of granule fragments was also determined in each sample.

Swelling power and solubility were determined by a modification of Schoch's method (69). Samples were removed from the amylograph bowl at appropriate intervals and immediately suspended in distilled water heated to the temperature of the suspension in the amylograph bowl. Each sample was diluted to 200 ml, transferred to a previously weighed centrifuge bottle, and centrifuged for 15 minutes at 2000 rpm. Aliquots of the supernatant were removed for the solubles determination, and the rest poured off as quantitatively as possible and saved. The centrifuge bottle and residue were weighed, the supernatant returned, and the suspension transferred to a blender and homogenized. Aliquots of this homogenized suspension were taken to determine the total solids present. The total solids, solubles, and swelling power were determined for each sample by the following equations:

$$\text{Total solids} = \frac{\text{weight of residue}}{20 \text{ ml aliquot}} \times 8 + \frac{\text{solubles}}{20 \text{ ml aliquot}} \times 2$$

$$\text{Solubles} = \frac{\text{weight of residue}}{\text{total solids}} \times 10$$

$$\text{Swelling power} = \frac{\text{weight of residue}}{\text{total solids}}$$

The enzyme susceptibility of natural and defatted samples was determined by a modification of AACC Method 22-15 (1). Samples of the starch were suspended in appropriate amounts of distilled water and heated for varying intervals up to three hours in a water bath. The paste was diluted and transferred to a 250 ml volumetric flask, made up to 250 ml, and 10 ml aliquots of

this suspension were removed for the determination of maltose. These aliquots were added to 46 ml of acetic acid-acetate buffer (pH = 4.6-4.8) and 5 ml of beta-amylase solution (4 mg/5 ml) was added. The reaction mixture was held at 30°C in a water bath and 10 ml aliquots of the reaction mixture were removed each hour for a period of three hours and transferred to a test tube containing 10 ml of 0.1 N ferricyanide, and maltose was determined according to AACC Method 22-15 (1).

Iodine affinities of the samples were determined according to Lansky et al. (32).

RESULTS AND DISCUSSION

Appearance of the Secondary Viscosity Peak. The amylograph viscosity curves obtained for each of the six starches employed in this study are illustrated in Figures 1A and 1B. Two different patterns are immediately evident. The waxy starches and potato starch exhibited an initial viscosity peak followed by an extensive breakdown, and the viscosity continued to decrease upon prolonged heating. Thick-boiling corn starch, wheat starch, and grain sorghum starch, all exhibited viscosity characteristics that are similar to each other. Under optimal conditions of concentration and temperature, each of these starches showed an initial viscosity increase, little breakdown after attaining the primary peak, and a secondary viscosity increase after varying periods of heating. The grain sorghum starch produced the most prominent secondary peak and required the shortest heating period to reach it, while wheat starch exhibited the least prominent

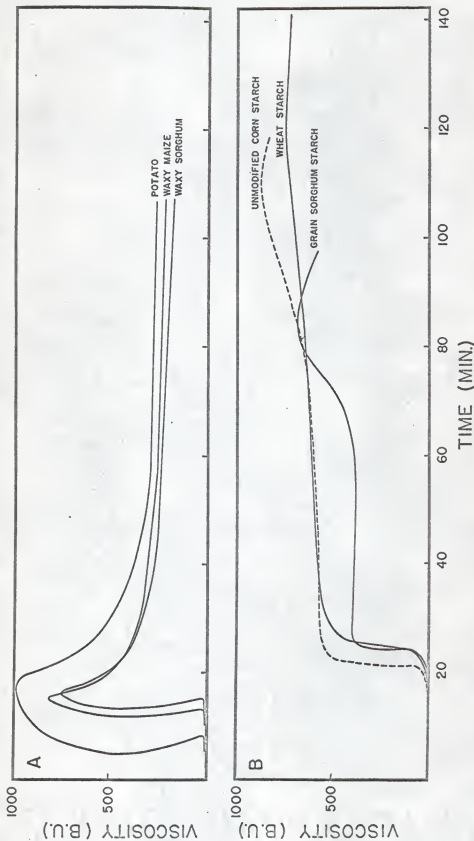


Figure 1. Amylograph viscosity curves of various starches heated from 50°C to elevated temperatures and held for varying periods of time.

secondary peak and required the longest heating period to attain it.

A microscopic examination of the unmodified corn starch reveals on a granular level, what is being measured by the amylograph on a much larger scale. The results of the microscopic examination are given in Figure 2. The initial rise in viscosity, as measured by the amylograph, was reflected in a corresponding rise in the average diameter of the individual granules. The viscosity and average diameter then went through a period of relatively little change before both exhibited a secondary increase. Up to this point both curves are similar, and the two viscosity increases may be attributed to the corresponding changes in the size of the individual granules. The degree of fragmentation remained low during this period.

After the secondary increase the viscosity reached a peak and began to decrease throughout the remainder of the heating period. The granule diameter, however, continued to increase, though at a reduced rate. At the point where the viscosity reached its peak and began to decline, a marked increase occurs in the degree of granular fragmentation from approximately 3 percent to more than 10 percent between 80 and 120 minutes of heating time. The observed viscosity decrease may be attributed to the increased breakdown of the individual granules, with a consequent decrease in the total volume occupied by the granules.

Effect of Natural Lipids. As the fatty materials, either naturally associated with the starch or added to it, are known to affect the swelling and solubility of the starch. Figures 3A,

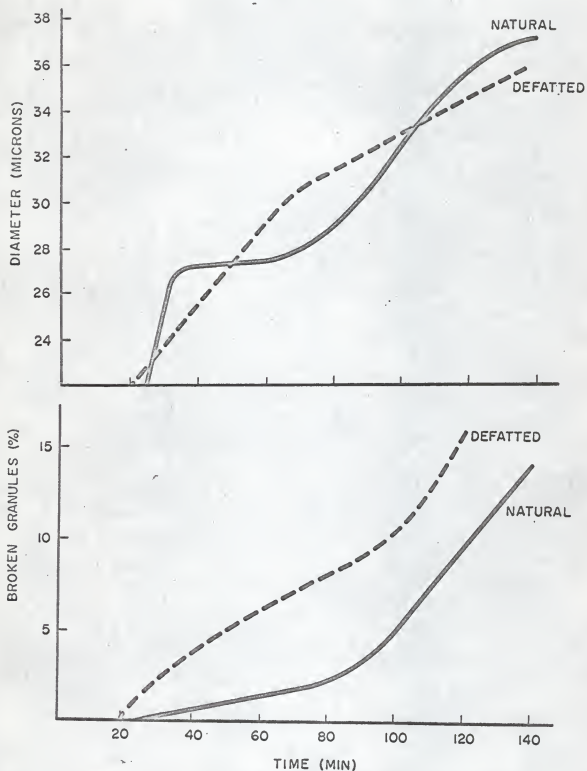


Figure 2. Average granule diameter and percent broken granules in natural and defatted corn starch at holding temperature of 90°C.

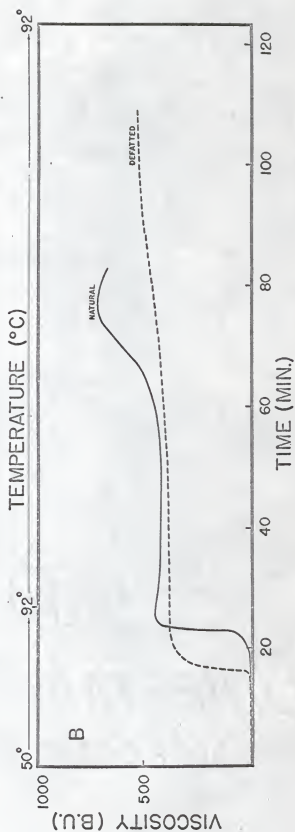
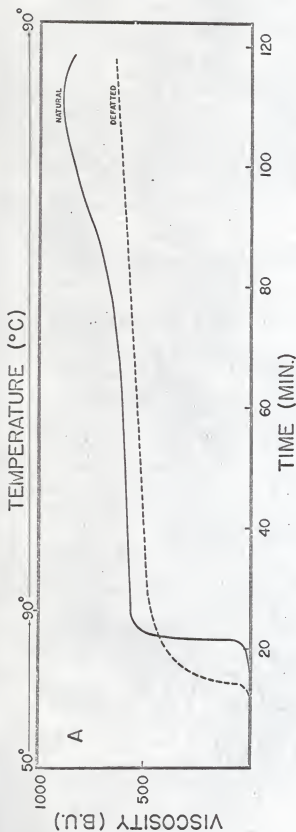


Figure 3. Effect of defatting natural starches on the secondary viscosity peak. A. Unmodified corn starch, 4.3 g starch/460 ml water. B. Grain sorghum starch, 4.0 g starch/460 ml water.

3B, and 4A show the effect on the amylograph viscosities of defatting corn, sorghum, and wheat starches, respectively. It is evident that fat removal caused the disappearance of the secondary peak from corn starch, greatly reduced it in grain sorghum starch, and had little effect in wheat starch, which originally exhibited only a very slight peak. The defatted starch in each case had a lower apparent gelatinization temperature, and at temperatures below 90°C attained a greater degree of swelling than the natural samples. These results agree with the swelling power results obtained by Gray and Schoch (20) who have shown that the presence of fatty materials has an inhibitory effect on the initial gelatinization of the granules, but that this inhibition is reversed above 90°C and the granules then swell freely.

Watson (76) reported that defatting the starches by the Soxhlet procedure had little or no effect on the secondary viscosity peak. However, results obtained in this study indicate that although the Soxhlet procedure was not as efficient as the reflux procedure in causing the disappearance of the secondary peak, there was nevertheless a substantial decrease.

Figure 2 also illustrates the results of a microscopic examination on a defatted sample of unmodified corn starch. As in the natural sample, the initial viscosity increase was reflected in an increase in the average granular diameter. However, in this case, the average diameter showed no secondary increase. The diameter increased linearly for the first hour of heating and at a reduced rate for the remainder of the heating

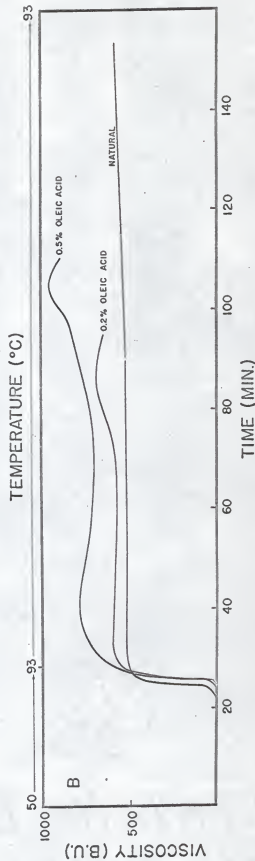
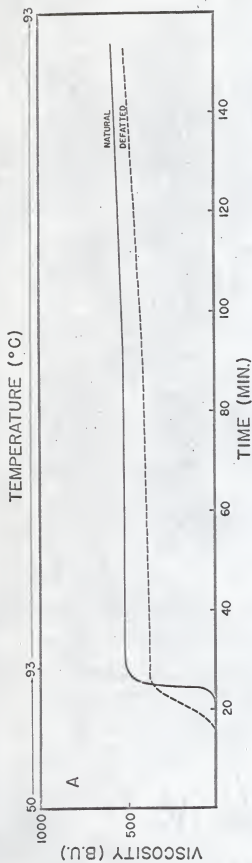


Figure 4. A. Effect of defatting wheat starch on the secondary viscosity peak. B. Effect of adding oleic acid to natural wheat starch.

period. The viscosity curve exhibited the same trend. After the initial increase, the viscosity continued to rise at a reduced rate over the rest of the heating period. The degree of granular breakdown was nearly linear throughout the entire heating period. The amylograph viscosity of the defatted sample was higher than that of the natural sample only at temperatures below about 90°C. Above this level, the natural sample showed a higher viscosity. The degree of granular breakdown was higher in the defatted sample at all points.

Effect of Added Fatty Acids. Since wheat starch exhibited the least prominent secondary peak, it was chosen for the re-fatting study. The procedure for the refatting (20) was designed to regenerate the fatty acid within the granule as it is thought to occur naturally, and not merely as a coating over the granular surface. Figure 4B illustrates the effect of adding oleic acid to natural wheat starch. Also included in this figure is the viscosity curve for normal, non-defatted, wheat starch. It is clear from the curves that the fatty materials present in the starch are of primary importance in the appearance of the secondary peak. The natural wheat starch exhibited no secondary peak, but the viscosity continued to increase slightly over the entire heating period. When 0.2 percent of oleic acid was added to the natural wheat starch, a very well defined secondary peak appeared, and this secondary peak was even more pronounced when an addition of 0.5 percent of oleic acid was made to the natural wheat starch. The addition of increasing amounts of fatty acid progressively increased the initial gelatinization temperature and the primary

viscosity peak.

The results, up to this point, indicate that the viscosity changes which occur in the different samples may be attributed to changes which take place in the individual granules, as observed by the microscopic examinations. The presence of the natural lipid material or the addition of fatty acids seems to be a necessary prerequisite for the formation of the secondary peak. The presence of these lipid materials also seems to increase the resistance of the granules to fragmentation. The microscopic data indicate that the natural granules are able to attain a greater degree of swelling without fragmentation than the defatted granules. The addition of oleic acid to natural wheat starch also gives this starch the ability to undergo greater swelling before fragmentation causes a decrease in viscosity.

Swelling Power and Solubility. The swelling power determination is essentially a measure of the amount of water held by the swollen granules and, as such, gives an indication of the degree of hydration of the starch. It is realized that the application of the large gravitational force which is involved in this determination may alter the shape and volume of the granules and hence lead to misleading results. The more rigid granules will not pack as closely as the less rigid ones, leaving more intergranular space to be filled with water. The less rigid granules will be deformed by the increased gravitational force and will occupy the intergranular space and thereby decrease the apparent swelling power of the sample. These less rigid granules may also be

compressed and lose volume, again decreasing the apparent swelling power.

The results of the swelling power and solubility determinations on natural and defatted corn starch are given in Figures 5 and 6. The swelling power curve of the natural sample exhibited a secondary increase in the amount of water held within the swollen granules, which corresponded in time to the secondary viscosity increase. The defatted corn starch exhibited no such secondary increase, the swelling power of this sample, after an initial increase, remaining constant over the entire heating period. The decrease in swelling power exhibited by the natural sample after attainment of the secondary peak is not believed to be attributable to a decrease in hydration, but to the physical breakdown of the granular structure and consequent decrease in the volume of the residue.

The solubility results indicate that the defatted sample exhibited an initial increase in soluble material, which then levels off and remains constant throughout the remainder of the heating period. The natural sample exhibited an initial increase in soluble material which continued to increase at a reduced rate throughout the remainder of the heating period.

Effect of Holding Temperature. The effect of increasing the final holding temperature on the curve characteristics of grain sorghum starch are given in Figure 7A. The results agree with those obtained by Watson (76) for unmodified corn starch. The maximum hot paste viscosity increased slightly with increasing temperature, while the degree of breakdown increased progressively.

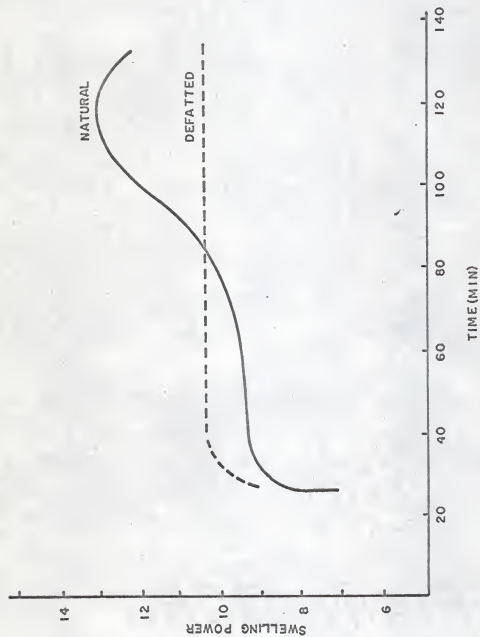


Figure 5. Effect of holding time at 90°C on the swelling power of natural and defatted unmodified corn starch.

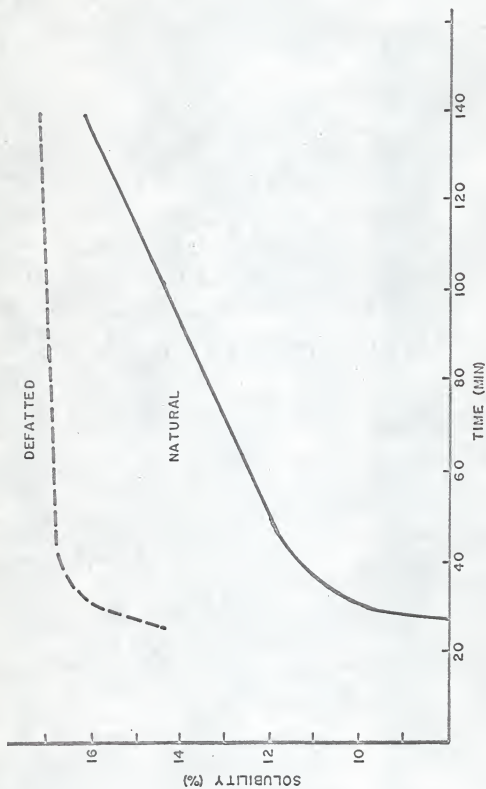


Figure 6. Effect of holding time at 90°C on the solubility of natural and defatted unmodified corn starch.

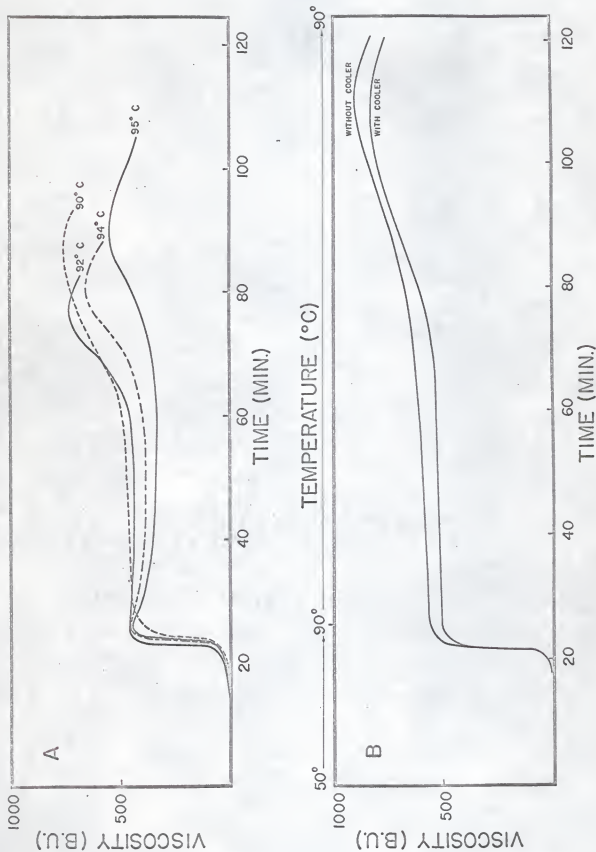


Figure 7. A. Effect of holding temperature on the secondary paste viscosity of rain sorghum starch (40 g starch/460 ml water). B. Effect of water evaporation on amylograph viscosity of unmodified corn starch (43 g starch/460 ml water).

The height of the secondary peak decreased as the temperature increased, but remained relatively constant with respect to the level viscosity portion of the curve. The time necessary to reach the secondary peak decreased between 90 and 92°C, but above this latter temperature the time increased. Watson (76) has shown that this same trend continued up to 97°C in unmodified corn starch.

The effect of temperature on the curve characteristics may be viewed as the result of a balance between the degree of swelling which the granules have undergone, and the degree of breakdown which has occurred. The degree of swelling which a granule undergoes in excess water becomes a function of the temperature. As the temperature increases, the amount of energy added to the system increases and the degree of swelling which a granule also undergoes increases. As the granules swell, they become increasingly soluble, the granular network is weakened, and the granules become increasingly susceptible to fragmentation. As the temperature increases, the degree of breakdown which occurs at the primary peak increases, and the number of granules which survive the initial hot-paste peak without fragmentation decreases. This requires that a greater number of the yet whole granules swell or that a greater degree of swelling occurs in the remaining whole granules, in order for the secondary viscosity increase to become evident. Thus, as the temperature increases, the extent of granular breakdown increases, and the secondary peak appears to be decreased and requires a longer heating period or the addition of more energy to become evident.

The increased time necessary to reach the secondary peak at 90°C is believed to be due to the lesser amount of energy which is added to the system at this temperature. The degree of granular breakdown is evidently lowest at this temperature, but it takes a longer heating time to add sufficient energy to produce the secondary swelling.

Effect of Evaporation. It has been suggested that the secondary viscosity increase might be caused by the increase in the concentration of the suspension due to the evaporation of water from the amylograph bowl during the long heating periods. In order to determine the effect of evaporation, the viscosity of several samples was measured with cold water running through the cooling coil on the cover of the amylograph bowl. Figure 7B shows that the reduced evaporation had no effect on the secondary peak of unmodified corn starch, either in the time of its appearance or on its relative height. The only apparent difference was in the height of the entire curve, it being lower when the cooling coil was in operation. This effect would be expected due to the lower concentration of starch in this sample. Determinations made with other starch samples produced similar results.

Effect of Concentration. The effect of increasing the concentration of the starch on the appearance and characteristics of the secondary peak in unmodified corn starch and grain sorghum starch is illustrated in Figures 8A and 8B, respectively. The effect seems to be identical in both cases. As might be expected, the height of the primary viscosity peak increased with increasing concentration and the apparent gelatinization temperature

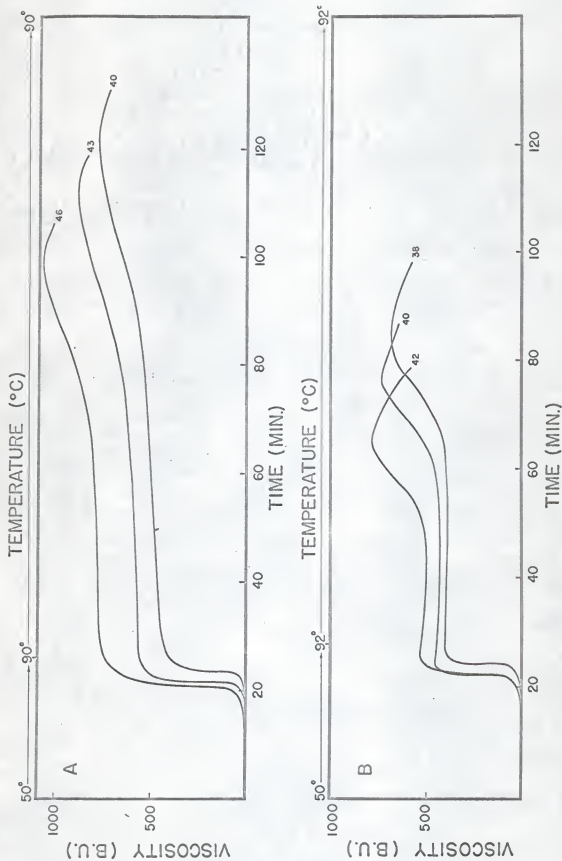


Figure 8. Effect of starch concentration on the secondary paste viscosity. A. Unmodified corn starch. B. Grain sorghum starch. Starch concentration in g starch/100 ml water.

decreased slightly. The secondary peak appeared after shorter periods of heating as the concentration increased, but its height relative to the constant viscosity portion of the curve remained fairly constant. Due to the greater number of intact granules which are present after the primary peak has been reached, less swelling must occur in each granule and hence less energy is required to produce the swelling necessary for the appearance of the secondary peak. The degree of breakdown after attaining the primary peak was slightly greater as the concentration increased; this might be attributed to the decrease in the freedom of movement available to the greater number of granules. The rate of breakdown after the secondary peak also increased as the concentration increased, but the differences were very slight.

Enzyme Susceptibility. The effect of the secondary swelling on the enzyme susceptibility of the starch is illustrated in Figure 9 where the ml of ferricyanide reduced is plotted against the heating time. The susceptibility is not affected by the secondary swelling in any case. The starch becomes completely available to the beta-amylase within the first seven to eight minutes after heating was begun, and no change occurred upon prolonged heating. A defatted sample showed increased availability, probably the result of its increased solubility and the increased availability of the linear fraction, due to the absence of complexing materials.

Effect of Carboxymethyl Cellulose. The effect of defatting the wheat starch and determining the amylograph viscosity under the conditions employed by Crossland and Favor (12) is illustrated

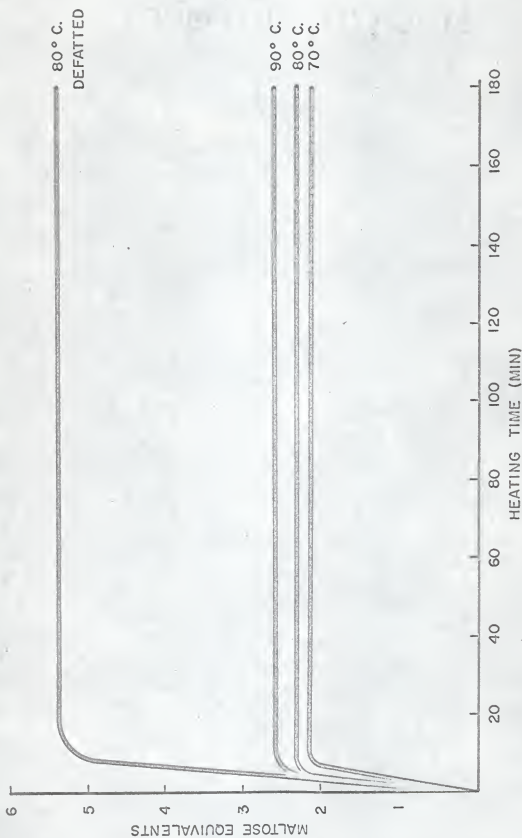


Figure 9. Enzyme susceptibility of natural and defatted unmodified corn starch.

in Figure 10. The starch was suspended in a 1 percent solution of carboxymethylcellulose and heated in the amylograph. The natural sample exhibited two distinct stages in the amylograph curve, while the defatted sample showed a higher initial viscosity and a less prominent secondary peak. This indicates that the secondary swelling reported here is at least partially dependent on the lipid material present in the natural starch.

SUMMARY AND CONCLUSIONS

Proposed Mechanism. The results of this study have established that the secondary viscosity peak is the result of a physical phenomenon involving the two-stage swelling of the starch granules. The microscopic data indicate that the swelling of the natural corn starch granule proceeds in two separate and distinct steps, and these data have been shown to mirror changes which occur in the swelling power and viscosity curves of the sample. It has been established that lipid material, either that normally present in the starch or that which is added, is necessary for the appearance of the secondary peak. It has also been shown that the presence of these fatty materials seems to impart resistance to breakdown and fragmentation to the granules.

Still another fundamental difference exists between the three starches which exhibited the secondary swelling and two of the three which did not. The data of Table 1 indicate that each of the former starches contain between 22.4 and 24.2 percent of the linear fraction, while the latter two are composed entirely of amylopectin. Potato starch is the only tuber starch included in



Figure 10. Pasting characteristics of natural and defatted unmodified corn starch in the presence of 1% carboxymethylcellulose.

this study, and its behavior is believed to be due to a fundamental difference in the molecular makeup of the granule and the absence of any appreciable amount of natural lipid material. This topic will be discussed further later in this report.

Table 1. Iodine affinity and amylose content of the starches used in this study.

Sample	: Iodine affinity : :(mg/100 mg starch):	: Percent amylose*
Thick boiling corn starch	4.72	23.6
Wheat starch	4.47	22.4
Potato starch	4.30	21.5
Grain sorghum starch	4.83	24.2

* Based on an iodine affinity of 20 mg/100 mg of pure amylose.

Gray and Schoch (20) have proposed a mechanism to explain the swelling behavior of various starches in the presence of surfactants, which entails the formation of a fatty acid-amylose complex and the subsequent breakdown of this complex at elevated temperatures. If a swollen starch granule is assumed to be a loose network of hydrated amylose and amylopectin molecules, held together by the more persistent micelles, and these micelles are tied together by the linear amylose molecules, then the degree of swelling which the granule may undergo will be determined, to a certain extent, by the distance which the micelles may move apart. This distance is, in turn, dependent on the length of the free amylose chains which connect one micelle to another. In a fat-free system, this distance will be the fully extended length of

the amylose molecules. In the presence of fatty acids, the free amylose chains which occur between micelles will be present as the fatty acid-amylose complex, which forces the amylose molecule to assume a helical configuration whose extended length is less than that of the free amylose chain. The degree of swelling which this granule will undergo is, therefore, reduced.

It has also been shown (20) that the fatty acid-amylose complex is unstable at elevated temperatures and above temperatures of about 90°C, the complex breaks down and the swelling power and solubility patterns show marked increases at this point. Thus prolonged heating of the complex, which occurs in the experiments conducted in this study, results in the eventual addition of sufficient energy to cause the relaxation of the complexing forces, and the amylose molecules are then freed to extend themselves to their full length. The overall result of this is the secondary swelling of the granule. The swelling power increase which corresponds to the secondary viscosity increase, might be a manifestation of the increased hydration of the amylose molecules as they are freed of the complexing agent.

The Case of Potato Starch. Potato starch presents a case which is not adequately explained nor understood by the proposed hypothesis. It contains about 21.5 percent of the linear fraction, which is nearly the same proportion of this component as occurs in the three cereal starches, yet it exhibits the swelling characteristics of the waxy starches.

There are several possible reasons for the anomalous behavior of potato starch. In the first place, natural potato starch

contains very little lipid material, when compared to the cereal starches (68). Schoch (65) has reported that native corn starch contains 0.6 to 0.7 percent fatty acid and wheat starch about 0.5 percent of mixed fatty acids and phospholipid. Natural potato starch contains only about 0.04 percent lipid material (15). So, the absence of any appreciable amounts of lipid material may be the reason that natural potato starch exhibits its anomalous behavior.

The addition of fatty acids to natural potato starch causes the viscosity behavior of this starch to become more like that of the normal cereal starches (20), but does not progress to the point of forming a secondary peak. Addition of 2.5 percent stearic acid to potato starch causes the apparent gelatinization temperature to be raised, and the maximum hot-paste viscosity to be decreased (20). The breakdown after the primary peak decreases with increasing fatty acid concentration, but the viscosity continues its decreasing trend during a one hour cooking period at 95°C. These results led to the conclusion that the fatty acid-amylose complex was formed in potato starch in the same manner as it formed in the cereal starches (20).

The fact that a fatty acid-amylose complex does form in potato starch, yet does not produce a secondary peak, thus requires a different explanation for its absence. On the basis of the solubility and swelling power curves of potato starch, Leach et al. (33) have concluded that the different patterns must be due to a difference in the molecular and granular structure of potato starch granules. It has been shown (34) that potato starch

is much less soluble at any given degree of swelling than other types of starch studied, which included waxy and normal sorghum and tapioca starches. This fact led to the conclusion that, in potato starch, the linear fraction was more intimately entangled within the molecular network of the granule than it was in other starch granules. There are thought to be fewer amorphous areas, and, on the basis of the great degree of granular breakdown at elevated temperatures, probably few areas of strong micellar association. It was suggested (33) that due to the higher equilibrium moisture of potato starch the hydrogen bonding occurs through water molecules rather than directly between adjacent starch molecules. The low temperature at which potato starch undergoes swelling was thought to be indicative of weak, though extensive, intermolecular bonding.

On the basis of this interpretation of the structure of the potato starch granule, it seems reasonable that a secondary peak might not appear, even in the presence of fatty acids. Upon addition of fatty acids to potato starch, the fatty acid-amylose complex forms in much the same manner as in the cereal starches. The initial gelatinization temperature is increased, the maximum hot-paste viscosity is decreased, and the breakdown after the initial peak is decreased (21). If the paste is held at elevated temperatures for long periods of time, the viscosity is seen to decrease slightly. During this heating time, energy is continually being added to the system and the intermolecular bonds which are holding the granule intact are being relaxed through the uptake of this energy. As these bonds are relaxed, the granular

structure is weakened and finally the granule is fragmented. This relaxation of the hydrogen bonding occurs before sufficient energy has been added to cause the breakdown of the fatty acid-amylose complex. By the time enough energy has been added to destroy the complex most of the granules have undergone fragmentation.

In the case of corn starch, there are strong micellar areas present which hold the granule intact, even at elevated temperatures. In this case, the bonds present in these micellar areas require a greater absorption of energy to be relaxed, than the bonds of the complex. As energy is added to the granule, the complexing forces are relaxed and the granule undergoes secondary swelling, and only at higher temperatures does fragmentation occur extensively.

In summary, the secondary viscosity increase found in the various starches studied here has been shown to depend on the lipid material naturally present in the starch or that added to it, and also to the amylose fraction of the starch substance. Waxy starches do not exhibit the secondary viscosity increase because they contain little or no amylose, and potato starch, which contains appreciable amounts of this fraction, does not exhibit a secondary viscosity increase due to a difference in its physico-chemical structure. The secondary viscosity increase has been shown to be the result of a secondary swelling of the granules as determined by the microscopic data and the swelling power results. Secondary swelling reported elsewhere in the literature seems to have the same basis as that studied here. Further work

in this area might include work with a greater variety of starches, use of other methods of analysis such as calorimetry, and a more comprehensive study of the effect of fatty materials on the physical properties of starch granules.

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AN INVESTIGATION INTO THE SECONDARY SWELLING
OF STARCH GRANULES

by

RICHARD ERNST PYLER

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An investigation into the viscosity behavior of five cereal starches and potato starch was conducted, with special attention being paid to the secondary viscosity increase noted in several of the cereal starches. The amylograph viscosity, swelling power, solubility, average granular diameter, and the degree of granular breakdown, were determined for each of the starches. In addition, the effect of the natural lipids and added fatty acids, the concentration of the starch, and the holding temperature, were determined.

A secondary viscosity increase was found in starch from normal varieties of corn, wheat, and sorghum, but not in waxy corn or sorghum or in potato starch. Removal of the natural lipids caused the disappearance of the secondary viscosity increase, and readdition of oleic acid caused its reappearance. The secondary viscosity increase corresponded in time to increases in the average diameter of the granules and the swelling power of the paste. The subsequent breakdown of the viscosity curve was attributed to an increase in the degree of granular fragmentation. Increasing the concentration of the starch caused a decrease in the time required to reach the secondary peak, while increasing the temperature caused an increase in the time to attain the secondary peak.

It was concluded that the secondary viscosity peak was due to a secondary swelling of the individual starch granules. The lipids are thought to be present as the amylose-fatty acid complex, which forces the amylose molecules to assume a constricted helical conformation, effectively tightening the molecular network

of the granule. Addition of energy destroys the complex, allowing the amylose molecules to expand, and the granules undergo a secondary swelling. Waxy starches do not exhibit the secondary swelling or viscosity peak since they do not contain any of the linear fraction. Potato starch, which contains approximately the same percentage of the linear fraction as the normal cereal starches, does not exhibit a secondary viscosity increase due to the absence of natural lipids, and to a fundamental difference in the associative forces which are present in this type of granule. Secondary swelling reported elsewhere in the literature is thought to be the same as that investigated in this study.